Early treatment of patients with malignant disease and liver or bone metastasis may increase their survival time. We have used the activity patterns of liver and bone isoenzymes of alkaline phosphatase (ALP), separated by agarose gel electrophoresis, to detect early metastasis. We studied ALP isoenzyme patterns in a background population of 101 patients with no evidence of any disease that might influence this pattern; a healthy reference population (n = 330); and the following three groups of patients: 143 with malignant disease, 47 with nonmalignant liver disease, and 22 with nonmalignant bone disease. Cutoff and predictive values of liver ALP, high-molecular-mass (high-M) ALP, and bone ALP were established for detecting liver and bone metastasis. The positive predictive value of liver and high-M ALP was higher than that of total ALP in detecting liver metastasis, but liver and high-M ALP did not enable us to differentiate between malignant and nonmalignant liver disease. Total ALP activity was of slightly more value than liver and high-M ALP in enabling us to rule out liver metastasis. From bone ALP activity we could not distinguish between nonmalignant bone disease and bone metastasis. The negative predictive value of bone ALP in the diagnosis of bone metastasis was low, but its positive predictive value was high and superior to that of total ALP.

Additional Keyphrases: cancer • liver • bone • metastasis

In patients with malignant disease, survival may be improved with early treatment (1). Symptoms of bone metastasis, and especially liver metastasis, appear late, and radiological diagnostic procedures, such as x-rays, ultrasound, and CAT scans are sensitive but costly and time-consuming. Simple laboratory techniques for the early detection of liver and bone metastasis in oncological patients are needed.

Alkaline phosphatase (ALP, EC 3.1.3.1) is a membrane-bound enzyme that can be resolved into tissue nonspecific ALP (liver/bone type), placental ALP, and intestinal ALP. Hepatocellular and cholestatic diseases, such as acute and chronic hepatitis, cirrhosis, carcinoma of the liver, metastatic carcinoma of the liver, and acute or chronic biliary obstruction, are all associated with increased liver ALP activity (2) and, frequently, with high-molecular-mass (high-M) ALP (3). High-M ALP is also known as fast-liver ALP (4), koinozyzme (5, 6), and bile ALP (7). Bone ALP is mark-

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4Nonstandard abbreviations: ALP, alkaline phosphatase; high-M, ALP, high-molecular-mass ALP; ROC, receiver–operator characteristics.

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probability (P) that a patient had metastasis (D+ = diagnosis present) when the ALP isoenzyme activity was above the cutoff value (T+ = test result positive):

$$P(D^+/T^+)=\frac{sens. \times prev.}{sens. \times prev. + (1- sens.) \times (1- prev.)}$$  (1)

and the probability (P) that this patient did not have metastasis (D− = diagnosis absent) when the ALP

$$P(D^-/T^-)=\frac{spec. \times (1- prev.)}{spec. \times (1- prev.) + (1- sens.) \times prev.}$$  (2)

For each variable, the cutoff value that combined maximal accuracy with maximal $P(D^+/T^+)$ and $P(D^-/T^-)$ values was selected (see Figures 3 and 5).

As an example, let us use an extreme cutoff value of 0 U/L for liver ALP: the sensitivity for detecting liver metastasis was 97% and the specificity was 1% [highest point of the ROC curve for liver ALP in Figure 1 (top panel)]; a cutoff value of 330 U/L yielded a sensitivity of 18% with a specificity of 100% [lowest point of the ROC curve for liver ALP in Figure 1 (top panel)]; and a cutoff value of 120 U/L combined a sensitivity of 67% with a specificity of 88%. The prevalence of liver metastasis being 27% in this population, the post-test probabilities were calculated as follows:

$$P(D^+/T^+) = \frac{0.67 \times 0.27}{0.67 \times 0.27 + (1-0.88) \times (1-0.27)}$$  (3)

$$P(D^-/T^-) = \frac{0.88 \times (1-0.27)}{0.88 \times (1-0.27) + (1-0.67) \times 0.27}$$  (4)

Thus, when the test is positive, the probability that the patient in this population has liver metastasis is 65%; when the test is negative, the probability that the patient does not have liver metastasis is 88%. The curve for liver ALP in Figure 2 (top panel) was obtained by changing the values for the prevalence of liver metastasis from 0% to 100% in equations 3 and 4. By this means we could judge the discriminating value of a test: the flatter the curves, the more insensitive the test, and the steeper the curves, the more sensitive the test.

Description of the Populations

We studied results for a total of 330 healthy adults and 313 patients for whom total ALP activity and ALP isoenzyme activity had been routinely determined between November 1989 and February 1990. Patients’ records were analyzed retrospectively.

Healthy reference population (n = 330). We previously studied the ALP isoenzyme patterns in 330 healthy adults. The age and sex distribution of this population was described earlier (12).

Background population (n = 101). As a background population we used a group of patients (ages 55 ± 33 years) without any of the conditions, such as lung embolism and chronic renal failure, known to cause an increase in total ALP or a change in ALP isoenzyme pattern. Patients with osteoporosis were not excluded (n = 13), because this condition is common in this age group and was usually not the only health problem. This population included 57 men and 44 women treated in 11 different hospital and outpatient departments. Their rec-
The most common sites of the primary tumors were breast (n = 36), lung (n = 30), and colon/rectum/sigmoid (n = 12), followed by ovary (n = 9), prostate (n = 8), kidney (n = 6), skin (melanoma and spinocellular carcinoma; n = 6), testis (n = 3), non-Hodgkins lymphoma (n = 3), bone (n = 3), bladder (n = 2), and other diverse sites (n = 8). No primary tumor was found in 3 patients; 6 had multiple tumors. Evidence of liver metastasis was obtained by liver echography or liver scan in 39 of these patients; 47 had bone metastases shown by x-ray or total body scan; and 19 patients had both liver and bone metastases. Some patients with liver or bone metastasis also had lung, brain, or other metastatic sites.

Results

The distribution and the percentiles of the ALP isoenzymes in the reference population and in the different patient groups are shown in Figure 1.

Background Population

Total ALP, liver ALP, and high-M₄ ALP activities were significantly increased in the background population compared with the healthy reference population. The distribution of bone ALP was greater in the background population than in the healthy reference population: both lower and higher activities were encountered. Only 1 of the 13 patients with osteoporosis had high concentrations of bone ALP (112 U/L). Of the 41 patients with respiratory problems, 9 had high concentrations of liver ALP or high-M₄ ALP, or both, compared with the healthy reference population. One patient from the intensive care department was intubated for several days and had increased placental ALP, detectable by electrophoresis.

Nonmalignant Liver Disease

Liver ALP and high-M₄ ALP were significantly increased in these patients compared with the background population. Bone ALP was also higher in the group with liver disease. None of the isoenzymes could be used to differentiate between the patients with liver disease and the patients with both malignant disease and liver metastasis.

Nonmalignant Bone Disease

Total and bone ALP were higher in these patients than in the background population. None of the isoenzymes could be used to distinguish between the group with bone disease and the patients with both malignant disease and bone metastasis.

Patients with Malignant Disease

Patients without liver metastasis. Concentrations of liver and high-M₄ ALP did not enable us to distinguish between the group of patients without liver metastasis and the background population.

Patients with liver metastasis. Total ALP, liver ALP, and high-M₄ ALP were significantly higher in the patients with liver metastasis compared with both the
isoenzymes were above these cutoff levels, a sensitivity of 70%, specificity of 88%, and accuracy of 84% were obtained for the whole group of patients. These values increased to 71% for sensitivity, 90% for specificity, and 85% for accuracy when patients with lung carcinoma were excluded.

It can be seen in Figure 3 that, with a pretest probability of 27% (the prevalence of liver metastasis in the population studied) and with cutoff values of 120 U/L for liver ALP, 10 U/L for high-M, ALP, and >92 U/L and >6 U/L for the combination of liver ALP and high-M, ALP, respectively, the P(D+/T+) values were 68%, 65%, and 70%, respectively, and the P(D−/T−) values were 88%, 88%, and 89%, respectively. When patients with lung carcinoma were not included, the incidence of liver metastasis and the P(D−/T−) values remained the same, whereas the P(D+/T+) values improved to 77%, 75%, and 73%, respectively.

Patients without bone metastasis. The activity of bone ALP did not enable us to distinguish between the patients without bone metastasis and the background population.

Patients with bone metastasis. Total ALP, liver ALP, and high-M, ALP were higher in the patients with bone metastasis compared to the background population. Bone ALP activity did not differ between these groups. Total and liver ALP were higher in the group of patients with bone metastasis than in the group of patients without such metastasis. The difference in bone ALP activity between these groups was not significant.

Fifteen of the 19 patients with both bone and liver metastasis had increased liver or high-M, ALP activities, or both, explaining the significantly higher liver and high-M, activities in patients with bone metastasis. However, 10 of the 28 patients with bone metastasis but without evidence of liver metastasis had increased liver or high-M, ALP activities, or both, and 6 of these 10 patients had lung carcinoma.

ROC curves were calculated for bone ALP and used in the detection of bone metastasis (Figure 4). For a cutoff value of 90 U/L, the sensitivity for detecting bone metastasis was only 33% for a specificity of 97%; the accuracy of the test was 76%. As shown in Figure 5, a pretest probability of 33% (the prevalence of bone metastasis in the population studied) and a cutoff value of 90 U/L yields 84% for P(D+/T+) and 74% for P(D−/T−).

Discussion

The technique we describe here for the detection of bone and liver metastasis in cancer patients is sensitive and reproducible and poses no major technical problems. The agarose gels are precast and the reagents are ready for use. Native serum and serum treated for a short period (5 min) with a polyclonal antiserum that reacted with placental ALP and intestinal ALP are usually run side by side. When bone ALP exceeds 50% of the total ALP activity, neuraminidase must be added to enhance the separation of liver and bone ALP (10, 12). The whole procedure, including scanning of the gels, takes about 3 h for 10 samples. The cost of the test is low compared.
with the costs of radiographic and scintigraphic procedures and various other tests, such as CEA dosage with radionuclides (which in Belgium costs about twice as much as ALP electrophoresis).

The use of this method for determining the distribution of the different ALP isoenzymes according to age and sex in a healthy population was described previously (12). In the present study we found that, compared with the previous healthy reference population, a background population of patients with no evidence for liver or bone disease had nonspecific increases in liver, high-M, and bone ALP. This justified our use of this background population in the present study of isoenzymes in malignant disease.

Electrophoresis of a serum sample from one of the patients in this background population revealed a placental ALP fraction. With the Isopal system, the major placental ALP fraction comigrates with bone ALP (10); it is differentiated from the intestinal variant ALP fraction by its sensitivity to neuraminidase and its resistance to heat (65 °C, 10 min). In this patient, the placental nature of the abnormal fraction was confirmed by its reaction with a monoclonal antibody to placental ALP. Specific dosage of placental ALP determined with an enzyme-linked immunosorbent assay (more sensitive than the electrophoretic procedure) was not routinely performed in this study. The finding of placental ALP in a patient on artificial respiration and with no signs of malignancy is not surprising, because increased serum concentrations of this isoenzyme are found in several lung diseases. Such increases are thought to be caused by an increase in alveolar epithelial and endothelial permeability with leaking of placental ALP, produced by type I pneumocytes, into the circulation (13, 14).

Bone ALP was increased in the group with liver disease. This might be explained by inadequate intestinal absorption of vitamin D, in cases of prolonged biliary obstruction or cirrhosis, or by an impaired vitamin D metabolism of the liver, resulting in the development of osteomalacia (15).

Because serum samples are taken at regular intervals during the follow-up of all patients with malignant disease in our hospital, and because we did not select the patients but processed the results from consecutive routine samples, the 143 oncological patients we studied can be considered representative for the population of the oncological department in our hospital. A review of the medical records for this population showed that the prevalences of liver and bone metastases were 27% and 33%, respectively.

Liver Metastasis

High-M, ALP consists of liver ALP attached to fragments of the liver cell membrane; variously sized vesicles are visible in these fragments when examined in the electron microscope (6). High-M, ALP is present in the serum of patients with well-defined biliary obstruction (5, 16, 17) and in patients with hepatic metastasis of solid tumors (18, 19). Viot et al. (20) studied 202 patients with different malignant conditions by cellulose acetate electrophoresis. These investigators concluded that high-M, ALP was highly correlated with the presence of liver metastasis and that assays of this ALP isofrom provided greater specificity (90%) and sensitivity (97%) than those of γ-glutamyltransferase and total ALP; however, they did not include a large variety of benign pathologies in their study. Nishio et al. (18) examined the diagnostic value of high-M, ALP separated by cellulose acetate electrophoresis in 126 lung cancer patients and 15 controls with benign respiratory diseases, and concluded that this isoenzyme was most useful in patients with small cell lung cancer, which often shows widespread hepatic metastasis. They found a sensitivity of 71%, a specificity of 89%, and an accuracy of 66% for high-M, ALP. Mayne et al. (21) studied total, liver, and high-M, ALP in 140 patients with...
breast cancer. They found lower sensitivities than the other authors, but, as might be expected, higher specificities and an accuracy of 85% for total ALP and of 88% and 86% for liver and high-M₄ ALP, respectively. They attributed the low sensitivity of the isoenzymes to the diagnosis of liver metastasis occurring at an earlier stage compared to other studies (more false negatives).

The sensitivity, specificity, and accuracy of high-M₄ ALP for detecting metastasis in our study were not as high as those found by Viot et al. (20) and Nishio et al. (18). High-M₄ ALP was definitely better than total ALP and only slightly better than liver ALP for detecting liver metastasis. Lower cutoff values could improve sensitivity but also resulted in a lower accuracy and straighter $P(D^+/T^-)/P(D^-/T^+)$ curves, parameters that were not studied by the other authors. Positive predictive values of liver and high-M₄ ALP increased slightly when lung cancer patients were excluded. This might be explained either by the presence of liver metastasis not detected by echographic and scintigraphic procedures or by nonspecific increases in liver and high-M₄ ALP in lung cancer without liver metastasis. The presence of increased liver and high-M₄ ALP activities in some patients with benign lung disease (see background population) supports the latter hypothesis. Although combining the activities of liver and high-M₄ ALP yielded the best results for detecting liver metastasis, total ALP proved to be more useful for ruling out liver metastasis.

Bone Metastasis

The differences in total, liver, and high-M₄ ALP between the patients with and without bone metastasis were due to the presence of liver metastasis in 19 of the patients in the group that was positive for bone metastasis.

Determination of bone ALP activity did not differ significantly between the group that was positive for bone metastasis and the group that was negative for this condition. Because bone ALP is an indicator of osteoblastic activity, it is unlikely to increase in cases of osteolytic bone metastasis (e.g., most breast tumors). Thus, a normal bone ALP activity has only a very limited value in ruling out bone metastasis, whereas increased bone ALP activity is of value in detecting osteoblastic metastasis.

None of the ALP isoenzymes enabled us to distinguish between benign liver disease and liver metastasis or between benign bone disease and bone metastasis. Therefore, when liver, high-M₄, or bone ALP is increased without obvious cause, malignancy should be ruled out by further diagnostic tests. Once malignancy has been diagnosed, determination of ALP isoenzyme patterns can provide a noninvasive test for monitoring a patient between radiographic and scintigraphic procedures. An increase in the activity of liver, high-M₄, or bone ALP above the aforementioned cutoff values in a patient with malignant disease without previous signs of metastasis should also indicate the need for further evaluation of the patient.

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References


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